

Quantification of Microbial Communities in Hydrothermal Vent Habitats of the Southern Mariana Trough and the Mid-Okinawa Trough

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Abstract

The structure of microbial populations near chemosynthetic faunal communities of two geographically and geologically distinct deep-sea hydrothermal vent fields were quantitatively evaluated using catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH). The hydrothermal vent of the Southern Mariana Trough (SMT) was dominated by colonization of gastropods in the low-temperature diffuse hydrothermal fluid, whereas macrofauna in mixing zones of the Mid-Okinawa Trough (MOT) consisted of polychaetes, galatheid crabs, and bivalves. A quantitative comparison revealed that the microbial community of the SMT hydrothermal vent field is significantly different from that of the MOT and is strongly influenced by mixing conditions between reduced hydrothermal fluid and oxygenated seawater. In particular, a high proportion of *Epsilonproteobacteria* was found in the SMT hydrothermal fluid, which is composed of approximately 88 % seawater. In contrast, sulfur oxidizers in *Gammaproteobacteria* were most abundant near vent fauna habitats in the MOT. Our results suggest that the SMT hydrothermal environment is distinct from that of the MOT and affects the community structure of macrofauna and microbial flora.

Keywords

Deep-sea hydrothermal vent • Fluorescence in situ hybridization (FISH) • Hydrothermal mixing zone • Quantitative microbial community analysis

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6.1 Introduction

In a deep-sea hydrothermal environment, mixing between hot, reduced hydrothermal vent fluids and cold, oxygenated ambient seawater forms a steep physical and chemical gradient. Vent-derived electron donors and seawater-derived electron acceptors are a source of energy for chemolithotrophic growth of prokaryotes, both of free-living and symbiotic forms. Consequently, these microorganisms play a fundamental role in sustaining primary production in hydrothermal ecosystems, supporting the growth of heterotrophic microbial flora and fauna, and maintaining the diversity and population density, thus creating an oasis for life in the deep-sea “desert” (Corliss et al. 1979; Karl 1995).

The geochemical profiles of hydrothermal fluids fluctuate temporally and spatially, due to the complex inorganic

Table 6.1 CARD-FISH probes used in this study

Probe	Specificity	Sequence (5'–3') ^a	Target site ^b	FA (% vol/vol) ^c	NaCl (mM) ^d	References
EUB338 I	<i>Bacteria</i>	GCTGCCTCCCGTAGGAGT	16S, (338–355)	55	3	Amann et al. (1995)
EUB338 II	<i>Planctomycetales</i>	GCAGCCACCCGTAGGTGT	16S, (338–355)	55	3	Daims et al. (1999)
EUB338 III	<i>Verrucomicrobiales</i>	GCTGCCACCCGTAGGTGT	16S, (338–355)	55	3	Daims et al. (1999)
ARCH915	<i>Archaea</i>	GTGCTCCCCGCAATTCCT	16S, (915–934)	60	0	Amann et al. (1995)
ALF968	<i>Alphaproteobacteria</i>	GGTAAGGTTCTGCGCGTT	16S, (968–985)	30	3	Neef (1997)
BET42a	<i>Betaproteobacteria</i>	GCCTTCCCACTTCGTTT	23S, (1027–1043)	55	3	Manz et al. (1992)
GAM42a	<i>Gammaproteobacteria</i>	GCCTTCCACATCGTTT	23S, (1027–1043)	55	3	Manz et al. (1992)
DEL495	<i>Deltaproteobacteria</i>	ARTTAGCCGGYGCTTCCT	16S, (495–512)	55	3	Loy et al. (2002)
EP402	<i>Epsilonproteobacteria</i>	GAAAKGYGTCATCCTCCACG	16S, (402–423)	35	42	Takai et al. (2004)
CF319a	<i>Cytophaga-Flavobacterium</i>	TGGTCCGTRTCTCAGTAC	16S, (319–336)	55	3	Manz et al. (1996)
SUP05-187	SUP05	GGGCTCCTTTTCTCCATA	16S, (787–205)	55	3	Sunamura et al. (2004)

^aM = A or C, R = G or A, Y = C or T, W = A or T, K = G or T

^bPosition in the 16S or 23S rRNA of *E. coli*

^cFormamide concentrations in the hybridization buffer

^dNaCl concentrations in the wash buffer

reactions in the fluid conduit (Le Bris et al. 2006; Rogers et al. 2007; Zielinski et al. 2011). The fluctuation of geochemical composition in hydrothermal mixing zones may have a significant impact on the rate of microbial growth and metabolic activity, biogeochemical reactions, and the dominant microbial population. In particular, reduced compounds such as H₂, CH₄, HS[−], and Fe²⁺ are not only essential energy sources for chemolithotrophs but also factors that control the composition and density of the microbial community. Previous studies have investigated habitat components, for example, the boundary zone (interface) between hydrothermal fluid and ambient seawater, including low-temperature diffusive flow (Holden et al. 1998; Huber et al. 2002, 2003; Perner et al. 2007), chimney structures (Schrenk et al. 2003; Takai et al. 2001), and hydrothermal sediments (Nunoura et al. 2010; Teske et al. 2002; Yanagawa et al. 2013). These studies demonstrated the prevalence of *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Bacteroidetes*, *Firmicutes*, and various candidate divisions in the hydrothermal system. Due to fluctuations in the environmental conditions, microbial communities in hydrothermally influenced regions exhibit variability on various spatial and temporal scales, including large-scale intra- and inter-field variability (Flores et al. 2012; Huber et al. 2010; Nunoura and Takai 2009), short-term temporal and spatial variability (Perner et al. 2009, 2013), and spatial variability on a small scale of a few centimeters to meters (Nakagawa et al. 2005; Takai et al. 2004). However, most studies focused on the variability of specific microbial populations in the zone adjacent to hydrothermal vents. To better understand microbial

population dynamics and their relation to hydrothermal environments in terms of chemistry and temperature, a quantitative approach to whole microbial members is necessary.

Despite the data accumulation of microbial diversity based on 16S rRNA sequencing, relatively few studies have conducted a quantitative analysis of microbial community composition in hydrothermal mixing zones. Fluorescence in situ hybridization (FISH) is one of the most reliable methods for quantifying microbial populations and can provide important information regarding the spatial distribution, diversity, abundance, and composition of microbial communities in the environment. Here, we describe the microbial composition of regions where reduced hydrothermal fluid and oxygenated seawater mix using catalyzed reporter deposition FISH (CARD-FISH). The hydrothermal fluid samples were collected around the Southern Mariana Trough (SMT) and the Mid-Okinawa Trough (MOT) in the western Pacific, two geographically and geologically distinct hydrothermal vents. The zonation of hydrothermal vent fauna (gastropods, polychaetes, galatheid crabs, and mussels) was used as a potential environmental indicator of physical and chemical gradients formed by the mixing of reduced hydrothermal fluid and oxygenated seawater. CARD-FISH, using nine oligonucleotide probes, identified several major phylogenetic groups including *Bacteria*, *Archaea*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, the *Cytophaga-Flavobacterium* cluster, and the SUP05 group of gammaproteobacterial sulfur oxidizers (Table 6.1) as components of the microbial community

associated with hydrothermal fluids and nearby vent fauna. These results increase our understanding of meso-scale (tens of centimeters to meters) spatial variation in microbial populations and long-term (year-scale) microbial dynamics.

6.2 Materials and Methods

6.2.1 Sampling Sites and Sample Collection

The hydrothermally influenced fluids were collected around the shimmering water that emerged through the gastropod patches (Snail site: 12°57.190'N, 143°37.165'E, water depth: 2,850 m) at the center of the spreading back-arc basin of the SMT (for a detailed description, see Toki et al. Chap. 45), and around the hydrothermal mixing zones within the large sulfide mound (North Big Chimney: 27°47.451'N, 126°53.805'E, water depth: 982 m) in the Iheya North Knoll hydrothermal field of the MOT (see Kawagucci Chap. 30). From the SMT hydrothermal field, 11 different shimmering fluids and 2 ambient seawater samples were collected during the YK03-09 cruise (14 Oct to 14 Nov 2003) and the YK05-09 cruise (24 Jul to 10 Aug 2005) of the R/V *Yokosuka* using the manned submersible *Shinkai 6500* and during the TN167A cruise (15–27 Mar 2004) of the R/V *Thomas G. Thompson* using the remotely operated vehicle (ROV) *ROPOS* (Table 6.2). The Snail site is located on a mound of pillow lava, and low-temperature diffuse fluids emanate from hydrothermal vents and are densely inhabited with the gastropod *Alviniconcha hessleri* around Marker #21 and Marker #24. A low-temperature hydrothermal vent site is also located to the south of the Snail site, where a microbial mat-like structure was observed in the vicinity of Marker #78. From the MOT, 3 hydrothermal fluids and 2 seawater samples were obtained during the NT05-03 cruise (14–20 Apr 2003) of the R/V *Natsushima* using the ROV *Hyper-Dolphin* (Table 6.2). Hydrothermal vents are surrounded by habitat zones of polychaetes, galatheid crabs (*Shinkaia crosnieri*), and bivalves (genus *Bathymodiolus*), in order of increasing distance from the vent, which may be due to the optimal hydrothermal mixing required to support the growth of these species.

The hydrothermal fluid samples were taken from each SMT and MOT sampling area using a bag sampling system, a rotary clean seawater sampling system (Kato et al. 2009), and a Niskin bottle (Table 6.2). The collected samples were immediately fixed for 6 h with 3.7 % neutralized formalin and stored at –80 °C for analysis onshore.

6.2.2 Chemical Characteristics of Hydrothermal Fluids

The onboard analyses, including potentiometric techniques, colorimetric methods, titration, and liquid chromatography were performed as described by Gieskes et al. (1991). The pH at room temperature was determined using a pH meter with a combined glass electrode (IWAKI, IW055-BNC). The pH meter was calibrated daily using JSCS buffer solutions (pH = 6.865 and 4.010). Alkalinity was determined by titration with hydrochloric acid. The endpoint was calculated by Gran plot, using a pH meter (Corning, M-250) as a potentiometer. The calibration was verified by analyzing IAPSO standard seawater (with a known alkalinity of 2.45 mM), and the analytical precision was estimated to be within 3 %. The concentrations of dissolved silica, ammonium ions, and hydrogen sulfide were measured with a colorimeter by classical methods (Hach, DR2010) using molybdenum blue, indo-phenol and methylene blue, respectively. The analytical precision was generally estimated to be within 3 % for the seawater analysis.

The nitrate concentration was determined onboard using a liquid chromatograph (Shimadzu, LC10AI) with a UV detector ($\lambda = 220$ nm, Shimadzu, SPD10AVP) following a modified protocol (Maruo et al. 2006). To eliminate interference from major anions such as Cl^- and SO_4^{2-} , a high-capacity anion exchange column (Tosoh, TSK gel-SAX, 4.6×50 mm) was used with a 1 M sodium chloride eluent at a flow rate of 0.8 ml/min. Chloride concentration was determined by Mohr's method, using silver nitrate solution as the titrant and chromate solution as the indicator. Sulfate concentration was determined in the onshore laboratory using an ion chromatograph (Dionex DX-100) after diluting the samples to a ratio of 1:300. The concentrations of major and minor cations (Na, K, Ca, Mg, Sr, B, Mn, and Fe) were determined in the onshore laboratory using a flame spectrophotometer (Shimadzu AA-680) and inductively coupled plasma atomic emission spectrometry (ICP-AES; Seiko, SPS 1200AR) after diluting the samples adequately. The precision of these instrumental analyses was estimated to be within 5 % based on repeated analysis of a working standard.

All of the geochemical data from the SMT hydrothermal field are shown by Toki et al. (Chap. 45).

6.2.3 Catalyzed Reporter Deposition-Fluorescence In Situ Hybridization

The fixed samples were thawed and filtered through 0.2- μm -pore-size polycarbonate filters to isolate microbial cells.

Table 6.2 General description and CARD-FISH-based microbial community composition of the samples used in this study

Cluster ^a	Field	Site	Dive	Sample number ^b	Sampling location	Date	Temp. (°C)	Mg (mM)	Seawater content ^d (%)	Total cell count (cells/ml)	CARD-FISH-positive cells (% of total cell count)								
											EUB338	ARCH915	ALF968	BET42a	GAM42a	DEL495	EP402	CF319a	SUP05
Low-temperature shimmering	SMT	Mk#21	D792	RC2	Gastropods	2003.10.29	~79	45.3	89.0	7.8 × 10 ⁴	45.7	8.0	0.4	5.0	9.8	1.8	8.1	0.1	nd
	SMT	Mk#24	D902	RC1	Gastropods	2005.7.28	39.3	46.1	90.6	1.9 × 10 ⁵	51.5	0.0	2.5	15.3	10.2	5.9	12.1	3.0	nd
	SMT	Mk#24 ^c	R773	RC5	Gastropods	2004.3.20	41	44.2	86.8	3.0 × 10 ⁵	51.2	0.3	0.0	13.7	8.4	0.7	11.4	0.0	nd
	SMT	Mk#21	R780	RC1	Gastropods	2004.3.26	50.1	43.9	86.2	1.8 × 10 ⁵	54.7	1.8	3.9	14.6	22.9	4.3	10.8	1.8	nd
	SMT	Mk#78	R775	RC3	Microbial mat	2004.3.22	23.3	41.1	80.7	1.2 × 10 ⁵	51.3	26.3	0.0	8.6	24.0	0.0	4.2	0.0	nd
Collected in 2005	SMT	Mk#78	R775	RC4	Microbial mat	2004.3.22	58.1	48.2	94.7	1.5 × 10 ⁵	45.1	12.4	0.0	13.2	16.1	8.2	0.0	0.0	nd
	SMT	Mk#21	R780	RC6	Gastropods	2004.3.26	50.9	44	86.4	2.2 × 10 ⁵	64.1	2.5	3.8	12.7	26.3	12.7	12.4	1.7	nd
	SMT	Mk#24 ^c	R773	RC4	Gastropods	2004.3.20	48.7	45.3	89.0	1.7 × 10 ⁵	58.1	10.3	0.9	11.6	20.1	4.0	20.3	0.1	nd
	SMT	Mk#24	D789	RC2	Gastropods	2003.10.26	~110	40.7	80.0	5.2 × 10 ⁴	62.1	15.7	17.5	7.1	21.8	1.5	2.7	3.8	nd
	SMT	Mk#24	D904	RC3	Gastropods	2005.8.2	74.5	44.4	87.2	1.3 × 10 ⁵	65.1	10.7	5.0	46.2	7.8	2.6	1.5	0.7	nd
Seawater-dominant	SMT	Mk#24	D904	RC5	Gastropods	2005.8.2	111.4	39.3	77.2	6.4 × 10 ⁴	46.3	1.0	1.6	32.3	0.8	2.0	4.3	1.3	nd
	SMT	Mk#24	D789	N1	Ambient seawater	2003.10.26	3	50.9	100	2.5 × 10 ⁴	45.6	10.9	0.9	1.0	0.7	10.5	1.6	0.8	nd
	SMT	Mk#78	R775	N	Ambient seawater	2004.3.22	1.4	49.1	96.5	1.8 × 10 ⁴	31.5	5.3	0.4	0.0	0.0	6.2	0.6	0.1	nd
	MOT	NBC	D392	B2	Polychaetes	2005.4.15	8	48.7	92.5	9.3 × 10 ⁴	56.9	27.2	0.2	0.0	0.3	1.1	2.4	0.8	21.7
	MOT	NBC	D392	N	10 m above the vent	2005.4.15	nd ^c	49.2	93.3	1.0 × 10 ⁵	54.2	27.4	0.0	0.0	0.2	0.1	0.1	0.0	13.5
	MOT	NBC	D393	B3	Bivalves	2005.4.16	5	48.8	92.7	1.2 × 10 ⁵	57.0	nd	0.5	0.0	0.5	4.9	3.1	2.0	24.9
	MOT	NBC	D394	B2	Galatheids	2005.4.17	nd	49.3	93.5	1.9 × 10 ⁵	60.3	nd	0.6	0.0	1.2	8.5	4.9	1.7	20.5
	MOT	NBC	D393	N	1 m above the vent	2005.4.16	nd	44.5	84.5	1.4 × 10 ⁵	65.0	nd	0.3	0.0	0.0	0.6	0.2	1.2	18.7

^aClusters are defined in Fig. 6.3^bThe hydrothermal fluid samples were taken using a bag sampling system (B), a rotary clean seawater sampling system (RC), and a Niskin bottle (N)^cNot determined^dSeawater content (%) = $[\text{Mg}]/[\text{Mg}]_{\text{background}} \times 100$. $[\text{Mg}]_{\text{background}}$ in the SMT and MOT were estimated to be 50.9 mM (Toki et al. Chap. 45) and 52.7 mM (Nakagawa et al. 2005), respectively

CARD-FISH was performed according to a previously published protocol (Pernthaler et al. 2002) with slight modifications, as described below. Microbial cells on polycarbonate filters were permeabilized with lysozyme (10 mg/ml in 0.05 M EDTA and 0.1 M Tris-HCl buffer [pH 7.5]) for 70 min at 37 °C. The filters were then treated with 1 % H₂O₂ in methanol for 30 min at room temperature to deactivate the endogenous peroxidase. The microbial cells were then hybridized for 3 h at 35 °C in hybridization buffer containing a horseradish peroxidase (HRP)-labeled oligonucleotide probe (final concentration: 5 pmol/μl). The filter was then incubated in a washing buffer for 10 min at 37 °C. The oligonucleotide probes, formamide concentrations in the hybridization buffer, and salt concentrations in the washing buffer are listed in Table 6.1. The filters were further washed with 0.05 % TritonX-100 in 1× PBS (145 mM NaCl, 1.4 mM NaH₂PO₄, 8 mM Na₂HPO₄ [pH 7.4]) for 15 min at room temperature and then incubated in a 1:50 Cy3-labeled tyramide solution (TSA direct; PerkinElmer) for 10 min at 37 °C in the dark. The filter was then washed with 0.05 % TritonX-100 in 1× PBS and dehydrated with ethanol. The microbial cells on each filter were stained with 1 μg/ml of 4',6-diamidino-2-phenylindole (DAPI). A ProLong Antifade Kit (Molecular Probes) was used to prevent photobleaching. To estimate the cell density, more than 1,000 cells in at least 25 microscopic fields were counted for each filter.

6.2.4 Cluster Analysis of Microbial Community Composition by CARD-FISH

A comparison of the microbial community structures was performed by cluster analysis using the web-based program Black Box (<http://aoki2.si.gunma-u.ac.jp/index.html>). A clustering algorithm based on the Ward method (Ward 1963) was applied to the bacterial community composition as determined by CARD-FISH using the oligonucleotide probes EUB338 I-III, ALF968, BET42a, GAM42a, DEL495, EP402, and CF319a. To reflect differences in the quantitative compositions of the microbial communities, none of the parameters were normalized.

6.3 Results and Discussion

6.3.1 Quantitative Assessment of Microbial Community Composition by CARD-FISH

The chemical composition of the hydrothermal fluid was strongly affected by the degree of dilution in the ambient seawater. Estimates based on Mg concentrations suggest that the fluid samples contained a large amount of seawater, ranging from 77.2 to 94.7 % (Table 6.2). However, the

microbial community composition of the hydrothermally influenced samples was significantly different from that of the ambient seawater. The CARD-FISH with specific oligonucleotide probes determined taxonomic composition and population size of prokaryotes inhabiting the hydrothermal vent environment. Up to 65.1 % of the total cell count by DAPI staining hybridized with the bacteria-specific probes EUB338 I-III, while up to 27.4 % of the total cell count hybridized with the archaea-specific probe ARCH915 (Table 6.2 and Fig. 6.1). Approximately one-half to one-third of the total cell count did not hybridize to either the bacteria- or archaea-specific probes and had smaller cell size than the other cells. Members of the *Gammaproteobacteria* were abundant in most of the samples from the SMT, accounting for more than half of the bacterial cells (~26.3 % of the total cell count). In contrast, the most abundant group in the fluid samples collected at the SMT in 2005 (D904RC3 and RC5) was *Betaproteobacteria*, which represented approximately 75 % of the bacterial cell counts. Members of the *Epsilonproteobacteria* also constituted a significant fraction of the microbial cells in the SMT hydrothermal fluids, representing up to 20.3 % of the total cell count. The *Epsilonproteobacteria* are widely distributed in deep-sea hydrothermal environments that are microaerophilic or anaerobic. The *Epsilonproteobacteria* include various chemolithoautotrophs that are capable of using hydrogen and sulfide as electron donors, and oxygen, nitrate, and elemental sulfur as electron acceptors (Campbell et al. 2006). At the SMT hydrothermal field, the *Epsilonproteobacteria* dominantly inhabited zones where the seawater content was between 86.2 and 90.6 % (Fig. 6.2). Our findings suggest that these bacterial groups are not randomly distributed in the hydrothermal environments but have a preference for a specific ecological niche. The ALF968- and DEL495-positive cells were abundant in the D789RC2 and D780RC6 samples, respectively. However, relatively few of these cells were detected in the other samples. CF319a-positive cells had a very low detectability, accounting for less than 3.8 % of most samples. In the samples from the MOT, most oligonucleotide probes identified very few cells, with the exception of SUP05-187. The SUP05 group of sulfur oxidizers in *Gammaproteobacteria* was most abundant near vent fauna habitats in the MOT, as previously shown in the hydrothermal plume at the Suiyo Seamount (Sunamura et al. 2004). Sulfur oxidation would be one of the most feasible reactions in the hydrothermal environment of the MOT.

6.3.2 Spatial and Temporal Variations in the Composition of the Bacterial Community

While the 18 samples have different profiles in bacterial components, cluster analysis revealed that the bacterial

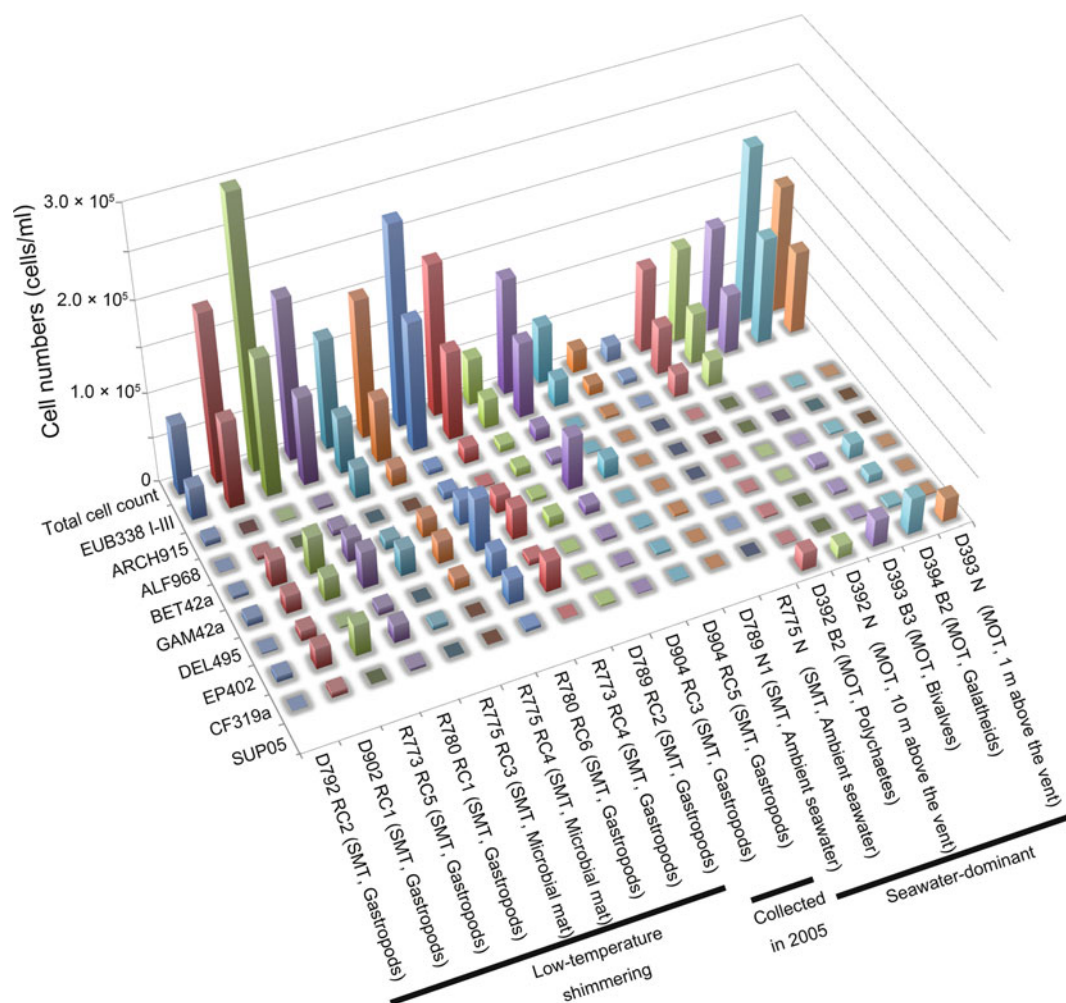
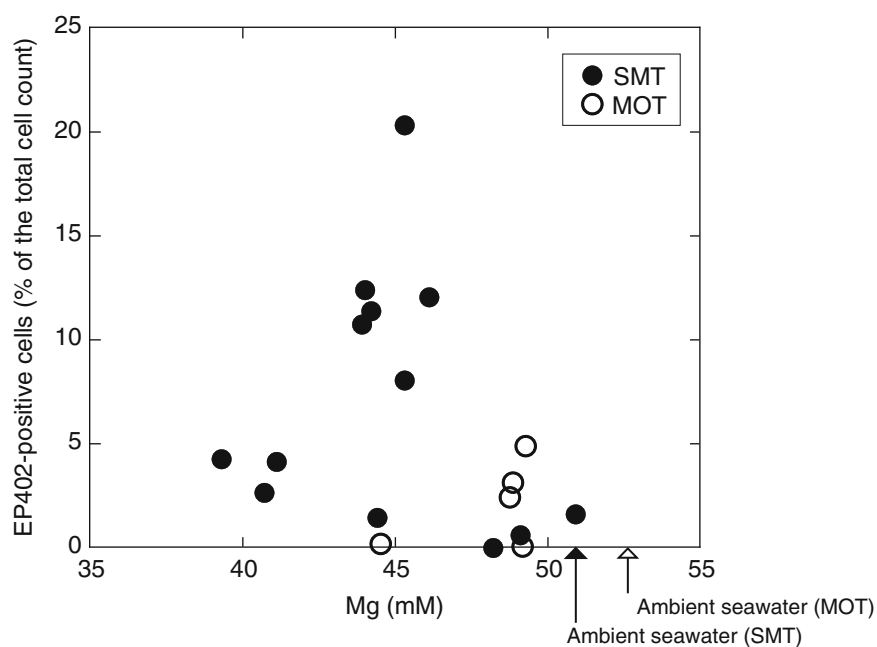


Fig. 6.1 Microbial community composition determined by CARD-FISH analysis

Fig. 6.2 The relative abundance of *Epsilonproteobacteria* in the mixed hydrothermal fluid and ambient seawater samples. The number of *Epsilonproteobacteria* cells was determined by CARD-FISH using the EP402 probe. *Solid circles*, hydrothermal fluid samples from the Southern Mariana Trough (SMT); *open circles*, hydrothermal fluid samples from the Iheya North Knoll hydrothermal field of the Mid-Okinawa Trough (MOT)



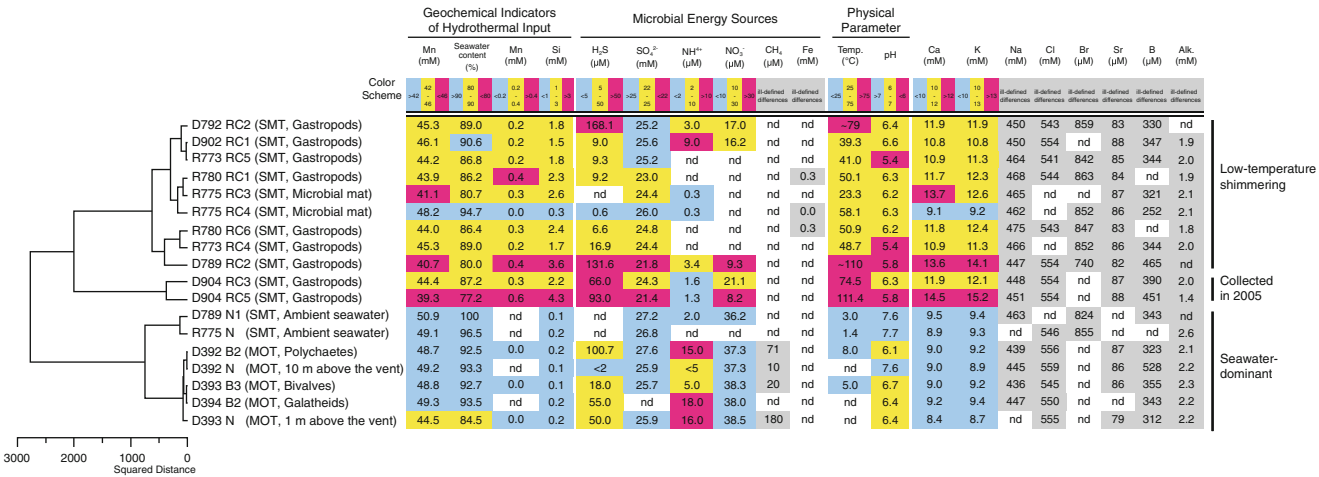


Fig. 6.3 Cluster analysis of the 18 bacterial communities and their environmental parameters in the deep-sea hydrothermal fluid and ambient seawater samples. The squared distance was calculated using the cell numbers as determined by CARD-FISH. The physical and

chemical compositions of the hydrothermal fluids are color-coded to represent the degree of hydrothermal input in descending order from red to yellow to blue. Gray indicates samples that did not exhibit significant differences

communities in the hydrothermal mixing zone formed a separate cluster from those in ambient seawater (Fig. 6.3). The chemical characteristics of the clusters suggest the following three distinguishable habitats conditions: (i) the seawater-dominant fluids composed of ambient seawater with a small portion of hydrothermal fluids, (ii) low-temperature shimmering, and (iii) the fluid samples collected at the SMT in 2005.

6.3.2.1 Seawater-Dominant Fluids

This cluster consisted of the ambient seawater samples and highly diluted hydrothermal fluids (Fig. 6.3). The average temperature during the sampling period ranged from 1.4 to 8.0 °C, and the Mg concentration ranged from 44.5 to 50.9 mM, corresponding to seawater proportions ranging from 84.5 to 100 %. Total cell densities ranged from 1.8 to 19×10^4 cells/ml (Table 6.2). CARD-FISH-positive cells for the probes of ALF968-, BET42a-, GAM42a-, DEL495-, EP402-, and CF319a were low in these samples (Table 6.2 and Fig. 6.1), possibly caused by the low expression of their ribosomal RNA or the dominance of other unidentified microbes.

6.3.2.2 Low-Temperature Shimmering

This cluster was composed of the samples from the SMT hydrothermal mixing zone (Fig. 6.3). The average temperature during the sampling period ranged from 23.3 to 110 °C, and the Mg concentration ranged from 40.7 to 48.2 mM, corresponding to seawater proportions ranging from 80 to 94.7 %. Total cell densities ranged from 5.2 to 30×10^4 cells/ml (Table 6.2). Though these shimmering fluid samples contained a substantial amount of seawater, the microbial community compositions in this cluster were notably different from the background seawater samples. The microbial

communities were mainly composed of BET42a-, GAM42a-, DEL495-, and EP402-positive cells (Table 6.2 and Fig. 6.1). A particularly high abundance of EP402-positive cells was detected in the sample of R773 RC4, comprising 20.3 % of the total cell count. However, EP402-positive cells accounted for less than 5 % of the total cell count in 12 of the 18 hydrothermal fluid samples (Fig. 6.2). The abundance of *Epsilonproteobacteria* in hydrothermal systems reported in previous studies is similar to the results found in this study (Nakagawa et al. 2006; Perner et al. 2007).

The microbial cell numbers were consistently high in the fluid samples from this cluster. Indeed, the total cell number in R773RC5 was 15-fold higher than in the ambient seawater samples (D789N1 and R775N). Furthermore, the microbial cells in the hydrothermal fluid samples were highly morphologically diverse, including rods, cocci, and filaments. A number of EP402-positive cells contained long filaments that were 3 μm wide and >100 μm in length. In contrast, the microbial cells isolated from the ambient seawater samples were mainly small cocci. Because the filamentous EP402-positive cells exceeded the other cells in size, their biogeochemical impact on the hydrothermal ecosystem would be larger compared to an equal number of smaller cells. Future characterization of these filamentous populations is needed to address this issue.

The microbial community composition in this cluster most likely reflects the difference in fluid chemistry. The emanating hydrothermal fluids are mixed with and cooled by ambient seawater. The low-temperature shimmering is enriched with reduced inorganic compounds (electron donors) from hydrothermal fluid and oxidants (electron acceptors) derived from ambient seawater. Therefore, a physicochemical gradient produced from oxic/anoxic

conditions and high/low temperatures around the hydrothermal vent may produce various habitat conditions to induce a phylogenetically, functionally, and morphologically diverse microbial community. The high proportion of *Epsilonproteobacteria* inhabiting oxygenated and low- to medium-temperature environments has been reported in previous studies (Huber et al. 2003; Nakagawa et al. 2005).

6.3.2.3 The Fluid Samples Collected at the SMT in 2005

The samples collected during the YK05-09 cruise in 2005 clustered separately from the other SMT samples (Fig. 6.3). The average temperature during the sampling period ranged from 74.5 to 111.4 °C, and the Mg concentration ranged from 39.3 to 44.4 mM, corresponding to seawater proportions ranging from 77.2 to 87.2 % (Table 6.2). Total cell densities ranged from 6.4 to 13×10^4 cells/ml (Table 6.2). This cluster was dominated by *Betaproteobacteria*, which accounted for ~46.2 % of the total cell count (Table 6.2 and Fig. 6.1). The dominance of *Betaproteobacteria* in habitats adjacent to gastropod aggregates may be supported by organic compounds excreted from gastropods, because heterotrophy is a dominant lifestyle in *Betaproteobacteria*. A similar temporal change in microbial community composition was previously observed in samples collected from the SMT borehole (Kato et al. 2009). This change may be due to long-term (year-scale) temporal fluctuation in the microbial community caused by a change in environmental factors.

6.3.3 Hydrothermal Habitats for Microbial and Macrofaunal Communities

Total cell densities ranged from 5.1 to 30×10^4 cells/ml in the hydrothermal fluid samples to 1.8 to 4.2×10^4 cells/ml in the ambient seawater samples (Table 6.2). Hence, the microbial cell abundance at the SMT was two- to ten-fold higher than in ambient seawater. In contrast, the total cell number in the hydrothermal fluid in the MOT was only twice that in the seawater samples. These data clearly indicate that the microbial community relies on hydrothermal energy input to the deep-sea where energy sources are typically scarce, and they reveal the regional variation and temporal fluctuation.

The stability of the hydrothermal activity is a primary factor to sustain the microbial and macrofaunal communities. The hydrothermal vents in the SMT are estimated to be stable over several years with regard to temperature and geochemical composition (Toki et al. Chap. 45). In contrast, the high-temperature venting activity in the MOT may create fluctuating habitats for both microorganisms and macrofauna due to vigorous mixing conditions.

6.4 Conclusions

In this study, we used a CARD-FISH-based quantitative approach to demonstrate that the microbial community composition of the SMT hydrothermal field is distinct from that of the MOT hydrothermal field. The SMT mixing zone harbored phylogenetically and morphologically diverse and abundant microbial populations compared to seawater-dominant samples. Although it was not possible to obtain data on the metabolism of microbial species detected by CARD-FISH (i.e., whether they were chemolitho-, mixo- or heterotrophic), the microbial community may include heterotrophs such as *Alphaproteobacteria*, *Betaproteobacteria*, and *Cytophaga-Flavobacterium* clusters. This finding may indicate that hydrothermal fluid components have an indirect impact on heterotrophic microbial populations. If so, future investigations should focus on the food web within the macrofaunal community. Our quantitative data will help predict the contribution of microbes to biogeochemical processes in deep-sea hydrothermal environments. Further research is needed to gain a better understanding of the functional role and biogeochemical impact of hydrothermal vent microorganisms.

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